Attorney's Docket No. 5051-451IP PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Li et al. Confirmation No. 8515
Serial No.: 09/914,020 Group Art Unit: 1635
Filed: December 31, 2002 Examiner: Epps Ford
For: *METHODS AND COMPOSITIONS FOR ALTERING MUCUS SECRETION*

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Linda D. Martin, Ph.D.
pursuant to 37 C.F.R § 1.132

I, Linda D. Martin, Ph.D., do hereby declare and say as follows:

1. I am a named inventor on U.S. Patent Application Serial No. 09/914,020 (*hereinafter* "the '020 application"). The work described in the '020 application was performed under the supervision and direction of me and my co-inventors Dr. Kenneth Adler and Dr. Yuehua Li.

2. I am an assistant professor at North Carolina State University. I received my Ph.D. from the Indiana University in 1993, and received postdoctoral training at Iowa State University from 1993 to 1994 and North Carolina State University from 1994 to 1998. I received my B.S. from Goshen College in 1986.

3. My research interests are in the area of molecular and biochemical mechanisms that affect human airway epithelial cell functions during inflammatory respiratory diseases.

4. I have read and understood the Office Action mailed on March 23, 2004, for the above-referenced application.

5. I, or people directly under my supervision have tested the effects of MARCKS-related peptides *in vivo* in mice.

6. In one of our studies, we elicited mucin hypersecretion by exposing ovalbumin (OVA) sensitized mice to aerosolized methacholine. This study included tests on three different groups of mice. One group of mice was treated intratracheally before the administration of methacholine with 50 µl MANS peptide (SEQ ID NO:1 in the '020 application) in saline at 10, 100 or 140 µM. A second group was pretreated with a missense peptide (random N-terminal sequence, hereinafter "RNS") at 100 or 140 µM. A third group was pretreated with saline alone. In this study, the amount of mucin released into tracheobronchial lavage fluid 30 minutes after methacholine exposure was determined by a mucin specific ELISA.

7. For mice treated with saline only, inhalation of methacholine caused an approximately five fold increase in mucin secretion. Figure 1A (attached) illustrates this effect and the effects of peptides on mucin secretion in mouse airways.

8. For mice pretreated with the MANS peptide before the administration of methacholine, we found that there was a concentration-dependent decrease in the overall response to the MANS peptide. Figure 1 illustrates the effects of peptides on mucin secretion in mouse airways. Administration of the MANS peptide in Figure 1A illustrates the reduction of mucin secretion in response to methacholine.

9. The mice that were pretreated with the missense RNS peptide in Figure 1 showed no mucus secretion effect at any of the doses provided. Fig 1A illustrates this lack of effect.

10. In the mice not exposed to methacholine, intratracheal delivery of a MANS peptide decreased basal constitutive levels of mucin secretion by 34% at 10μM and by 69% at 100μM. These studies are not included with this declaration.

11. We conducted similar studies in BALB/c mice. The results of these experiments were essentially the same as those observed in experiments with the OVA sensitized mice as disclosed in paragraphs 8-10 and Figure 1A. Figure 1B illustrates these results.

12. Figure 1C illustrates that the inhibitory effect of the MANS peptide was not restricted to a single secretory stimulus, as indicated by data showing inhibitory effects of MANS peptide on secretion induced by intraperitoneal delivery of 150 mg/kg pilocarpine.

13. For this study we have also performed histochemical staining with periodic acid–Schiff (PAS) and hematoxylin that revealed that airways exposed to OVA alone contained numerous mucin-filled goblet cells. Figures 2A and 2B illustrate mouse cells exposed to intratracheal saline and methacholine respectively.

14. Our studies have further illustrated that pretreatment with missense RNS peptide, as evidenced in Figure 2C had no effect on mucus secretion, whereas pretreatment with MANS peptide Figues 2D-2F markedly reduced the effects of methacholine in mucus secretion. Again this reduction improved in a concentration-dependent manner.

15. We have also shown that the association of MARKS with mucin granule membranes is inhibited by a MANS peptide (Figure 3). Figures 3A and 3B represent the use of gold-labeled immunostaining for MARCKS to provide ultrastructural evidence of the association between MARCKS protein and membranes of mucin granules. Figure 3C illustrates a western blot wherein lane 2 represent the control cells, lane 3 represents cells exposed to the missense RNS peptide and lane 3 represent cells exposed to 100μM of the MANS peptide. These figures illustrate the greatly reduced association of MARCKS protein with mucin granule membranes from cells exposed to the MANS peptide as compared to control or RNS peptide-exposed cells.

16. In summary, we have shown that a peptide directed against the conserved N-terminal region of MARCKS protein inhibits mucin release *in vivo* when instilled intratracheally into allergically inflamed mouse airways.

17. I do hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Linda D. Martin

Linda D. Martin, Ph.D.

8/18/04

Date

Attachments: Figures 1-3

Figure 1

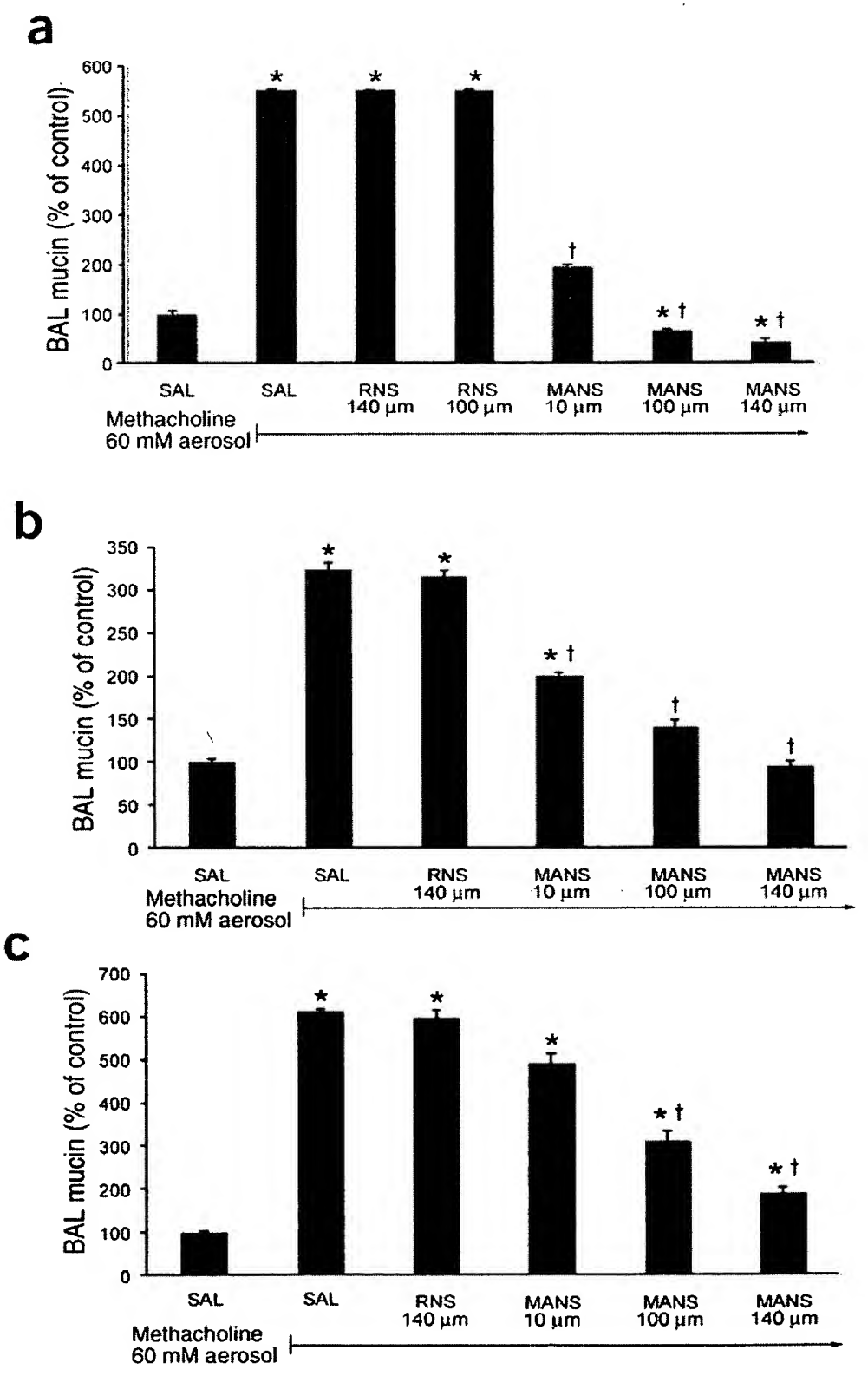

Figure 2

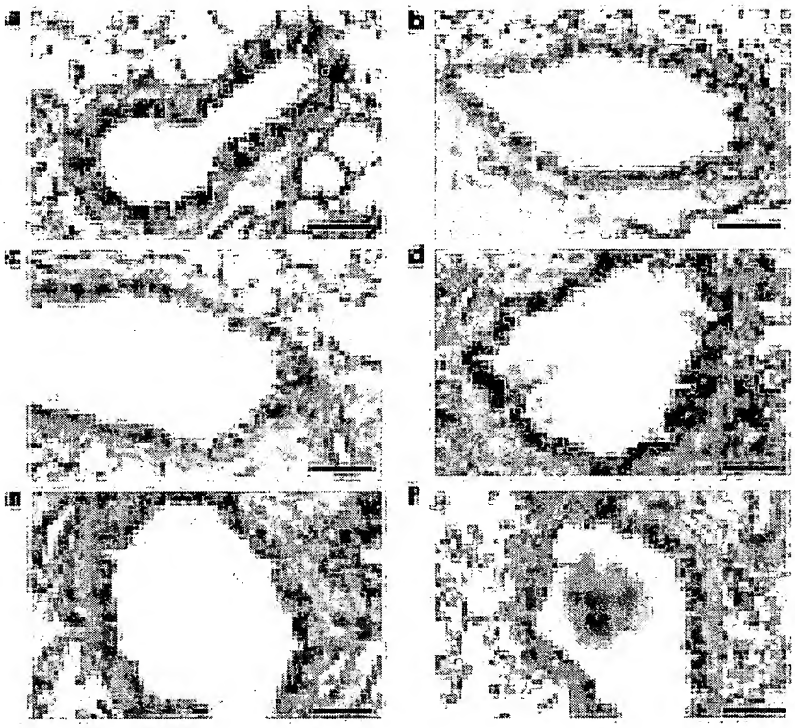

Figure 3